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Langerhans Cells: Functional Aspects Revealed by *in Vivo* Grafting Studies

J. WAYNE STREILEIN, M.D., GALEN B. TOEWS, M.D., AND PAUL R. BERGSTRESSER, M.D.

Departments of Cell Biology and Division of Dermatology, Department of Internal Medicine, Southwestern Medical School, Dallas, Texas, U.S.A.

We performed 2 types of experiments to reveal aspects of Langerhans cell function. In the 1st type, tail skins (mice) and cheek pouches (hamsters) were grafted heterotopically to the thoracic wall of normal recipients and were subsequently painted with immunizing doses of a chemical contactant, dinitrofluorobenzene. In the 2nd type, corneal tissue and skin irradiated with ultraviolet light were grafted to recipients immunogenetically disparate for class I (murine K/D) or class II (Ia) antigens to determine the ability of these grafts to elicit allograft immunity. We found that the inability of intact murine tail skin to support the induction of contact sensitivity was a property of the skin itself, not of the anatomical site. The inability to sensitize through cheek pouch was a property of the anatomical arrangement of the pouch in that an effective lymphatic drainage pathway did not exist. Although ultraviolet light apparently depleted body wall skin of ATPase-positive cells, it failed to rid skin of its capacity to express Ia antigens in a highly immunogenic way. Cornea grafts differing from their hosts across the *I* region alone of the major histocompatibility complex succeeded neither in inciting their own rejection nor in prejudicing the host's subsequent response to body wall skin allografts bearing the same *I* region antigens. These results are strong circumstantial evidence that Langerhans cells are the important epidermal factors promoting induction of contact hypersensitivity. Ultraviolet light appears to be an effective way in which to transiently perturb Langerhans cell function, but is not an effective means of removing Langerhans cells from skin. The results of the cornea graft experiments offer hope that, when effective means of erasing Langerhans cells from skin have been found, skin will be devoid of its capacity to promote contact hypersensitivity and to elicit allograft immunity directed at Ia antigens.

Experimental studies on epidermal Langerhans cells have utilized a variety of approaches. In the studies discussed in the 2 other papers we have presented here [1,2], we investigated Langerhans cell properties in 2 ways. First, we used a morphological approach to the quantification of Langerhans cells in the epidermis by taking advantage of the capacity of skin grafting, ultraviolet light (UVL) irradiation, and skin painted with noxious agents that induce contact hypersensitivity in order to alter normal Langerhans cell numbers and function. These studies led to the realization that under normal circumstances, considerable variation in Langerhans cell density and distribution exists among cutaneous surfaces. As a consequence of these morphological observations, we constructed several hypotheses that led to a 2nd avenue of investigation, in which we examined the functional properties of skin with natural and experimentally induced alterations in Langerhans cells. We used perturbed, but anatomically intact, skin to study the capacity of skin to promote contact hypersensitivity. The most interesting and provocative finding was that skin deficient in normal numbers and in distribution of Langerhans cells promoted the development of specific immunologic tolerance to a chemical contactant.

A 3rd approach to analyzing the physiological role of Langerhans cells was to utilize *in vivo* grafting techniques. We used grafts of normal body wall skin, skin from regions with unusual Langerhans cell densities, and skin in which Langerhans cells were putatively depleted. The results revealed that Langerhans cells are essential to, but not of themselves sufficient for, the induction of contact hypersensitivity and the induction and expression of allograft immunity.

MATERIALS AND METHODS

Animals

Mice of the following strains were obtained from our domestically maintained stocks: C57BL/6, A.AL, A.TL, A.TH, B10.A, B10.AQR, and B10.T(6R). The LSH hamsters were also obtained from our domestic stocks. Animals were between 2 and 4 mo of age at the time of experimentation. Panels of experimental and control animals were matched for age and sex.

Grafting

Full-thickness skin grafts were prepared from body wall skin and tail skin as described elsewhere [3]. Corneal grafts were carefully excised free of limbic tissue and placed, endothelium-side-down, on prepared graft beds [4]. Cheek pouch grafts were prepared from excised cheek pouches from which extraneous subdermal areolar tissue had been removed. Graft beds were created on the surfaces of the thoracic walls of recipient animals; precautions were taken to ensure that in each a panniculus carnosis layer was intact. Grafts were wrapped in plaster of Paris bandages, which were removed 8 days later. Grafts were inspected daily for evidence of rejection; rejection was judged to have occurred when all evidence of epidermis was gone from the graft surface. Median survival times were calculated according to the method of Litchfield and Wilcoxon [5].

Sensitization to Dinitrofluorobenzene

The method of dinitrofluorobenzene (DNFB) sensitization has been described elsewhere [6]. Two applications at 24-hr intervals of 25 μl of 0.5% DNFB in carrier (4:1, acetone:sweet oil) were placed at appropriate cutaneous sites. Four days later, dorsal surfaces of ears received 20 μl of 0.2% DNFB in carrier. The amounts of swelling, measured 24 and 48 hr later with a micrometer, were expressed as mean differences in inches ($\times 10^{-4}$). Positive controls consisted of normal animals whose abdomens had been painted twice with DNFB as described above and whose ears had been challenged. Negative controls received their 1st exposure to DNFB as an 0.2% solution placed on the ear.

Ultraviolet Light Irradiation

Ultraviolet light was administered according to a method described elsewhere [7].

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Reprint requests to: Dr. J. Wayne Streilein, Department of Cell Biology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235.

Abbreviations:
DNFB: dinitrofluorobenzene
SALT: skin-associated lymphoid tissue
UVL: ultraviolet light

EXPERIMENTS AND RESULTS

Two types of experiments were performed. First, skin with unusual numbers or densities of Langerhans cells was grafted heterotopically to the body wall of experimental animals. When completely healed in place, sensitization to DNFB was attempted through the grafted tissue. These experiments were designed to elucidate whether the inability of certain specialized cutaneous sites to promote contact hypersensitivity was a function of the skin itself or a property of the anatomical site where it resided. Second, skin either naturally or artificially deficient in Langerhans cells was assayed for its capacity to express transplantation alloantigens and to elicit transplantation immunity.

Capacity of Heterotropic Tail Skin Grafts to Support Induction of Contact Hypersensitivity to DNFB in Mice

Since normal mouse tail skin contained far fewer Langerhans cells than did body wall skin [7], and since immunizing amounts of DNFB painted on intact tail skin did not induce contact sensitivity [6], it seemed important to determine whether the failure resulted from an inherent property of tail skin itself, or, alternatively, whether there was something unusual about the anatomical site of the tail that precluded sensitization. To examine this matter, we placed large (approximately 20 × 25 mm) tail or body wall skin grafts on prepared beds of syngeneic C57BL/6 male mice. When the grafts had completely healed in place (30 days later), 25 μl of 0.5% DNFB in carrier was carefully applied to the surface of each graft. This procedure was repeated 24 hr later. Positive controls were normal male mice that received 2 similar paintings of DNFB on shaved, abdominal wall skin. Five days later the ears of each panel of animals, plus those of negative controls, were challenged with 20 μl of 0.2% DNFB. The ear swelling response was measured 24 hr later. We were gratified to find that grafts of body wall skin residing on the thoracic wall sustained and promoted the induction of contact sensitivity to DNFB, and did so at least as well as intact abdominal skin (34×10^{-4} inches versus 28×10^{-4}) (Table I). This important observation indicated that the process of grafting and healing with attendant scar information did not materially interfere with the afferent limb of sensitization to the chemical contactant placed on the grafted skin. By contrast, DNFB painted on tail skin grafted heterotopically to the thoracic wall elicited only marginal sensitization to the contactant. It seemed reasonable to conclude that we were observing a property of tail skin itself, not an artifact of the grafting procedure. Since DNFB painted on tail skin grafts resulted in a degree of sensitization only 36% that of the positive control value, we concluded that the tail skin, whether it existed at its anatomically intact location on the tail or was placed heterotopically, was fundamentally inefficient in inducing contact hypersensitivity. This finding formally excluded the possibility that our failure to sensitize mice via tail skin painted with DNFB was related to the anatomical location of the tail. Instead, tail skin itself was deficient in this property. (Among the many differences we found between tail and body wall skin, the anomalous densities and distributions of Langerhans cells were particularly striking.) We postulated that the failure of the tail skin to support contact hypersensitivity induction was related to the Langerhans-cell-associated abnormalities.

TABLE I. *Ear swelling response after dinitrofluorobenzene painting of heterotopic tail skins*

Immunization site	N	Ear swelling	
		× 10^{-4} inches	% Positive control
Body wall skin graft	4	34	121
Tail skin graft	5	10	36[a]
Abdominal skin[b]	4	28	100
Negative control	4	10	–

[a] Significantly less than positive control, $p < .01$.
[b] Positive control.

Capacity of Heterotopic Cheek Pouch Grafts to Support Induction of Contact Hypersensitivity to DNFB in Hamsters

Like the murine tail, the hamster cheek pouch exhibits aberrations of Langerhans cell number and distribution [7]. Moreover, the cheek pouch has long been known as an immunologically privileged site in which allo- and xenogeneic normal and tumor tissues can be successfully grafted for extended intervals [8]. Histological and functional studies have revealed that the cheek pouch is apparently devoid of a draining lymphatic network; a rather thick subdermal layer of loose, areolar tissue containing connective tissue of unusual consistency is thought to function as a barrier to the movement of molecules and cells from the cheek pouch epidermis to the deep subdermal lymphatics that drain to regional lymph nodes. Our studies have shown that cheek pouch epidermis, unlike body wall skin, contains a paucity of Langerhans cells in a distribution pattern that is randomly uneven.

Syrian hamsters, like mice, guinea pigs, and people, can easily be rendered hypersensitive to highly reactive contactants such as DNFB [9]. However, attempts to sensitize hamsters to chemical contactants through intact cheek pouch epidermis are fraught with real and hypothetical hazards. Although the pouch is easily evertable, one can not guarantee that DNFB painted thereon will not be swallowed by the animal once the pouch has been returned to its orthotopic site. Oral administration of contactants has been shown to be an excellent way to render guinea pigs and mice tolerant [10,11]. Thus, we elected to attack this issue by placing cheek pouch grafts heterotopically on the thoracic walls of syngeneic hamsters. When these grafts had healed in place and all evidence of acute inflammation had subsided, 2 applications of 0.5% DNFB spaced at 24-hr intervals were placed carefully on the graft surfaces. For controls, other hamsters that had received grafts of body wall skin on their thoracic cages received similar DNFB paintings on their grafts. At the time of application, the surface area of both types of graft was approximately 600 mm^2. The results of ear challenges of these animals are presented in Table II.

The body wall skin grafts in hamsters were as efficient as comparable grafts in mice at promoting the induction of contact hypersensitivity. The ear swelling of these animals (49×10^{-4} inches) was virtually identical to that of the positive controls whose intact abdominal skin had been painted (56×10^{-4}). By contrast, the mean ear swelling of the panel of cheek-pouch-grafted hamsters was only 32×10^{-4}, a value that exceeded the ear swelling of the negative control animal by only 7×10^{-4} inches. In 3 of the 5 animals with cheek pouch grafts, the ear swelling was indistinguishable from that in negative controls. However, in the other 2 animals, there was significant, albeit modest, ear swelling, an indication that a certain degree of hypersensitivity had been elicited. The cheek pouch grafts of these 2 animals simultaneously displayed an unexpected pattern of reactivity. Erythema developed in all DNFB-painted cheek pouch grafts within 24 hr of application. In the 3 instances in which no sensitivity emerged, the erythema faded within 72 hr. However, in the 2 animals that ultimately became sensitized (as measured by ear swelling) small necrotic areas were pro-

TABLE II. *Ear swelling response after dinitrofluorobenzene painting of heterotopic cheek pouch grafts*

Immunization site	N	Ear swelling	
		× 10^{-4} inches	% Positive control
Body wall graft	5	49	78
Cheek pouch graft	5	32	22[a]
Abdominal skin[b]	4	56	100
Negative control	4	(25)	0

[a] Of 5 animals, 2 had moderate ear swelling. We found violent inflammation in their cheek pouch grafts; this resulted in graft destruction 7 days after application of dinitrofluorobenzene. Ear swelling of 32 was significantly less than that of the positive control, $p < 0.01$.
[b] Positive control.

duced in the grafts by DNFB painting; a violent inflammatory reaction then developed in the cheek pouch grafts beginning 4 days after painting. The intensity of the reaction mounted during the next several days and finally culminated in complete graft destruction between days 7 and 8. As mentioned in another paper presented in this volume [1], cheek pouch grafts placed heterotopically rather quickly accumulated increased numbers of Langerhans cells in the epidermis. By day 30, at the time of DNFB painting, the density of Langerhans cells in these grafts approached that of the surrounding body wall skin. We interpreted the results of our cheek pouch painting experiments as follows: so long as DNFB painting of the cheek pouch epithelium failed to produce surface necrosis (and thereby preserved the "barrier"), contact sensitivity did not develop. However, when the toxicity of DNFB was sufficient to cause ulceration of the cheek pouch epithelium, systemic sensitization did ensue. The sensitivity expressed itself not only at the ear challenge site, where dilute DNFB was applied, but also at the original pouch graft site, where significant numbers of Langerhans cells and keratinocytes derivatized with DNFB remained. As a consequence, the effector limb of the immune response attacked the graft site and caused its destruction, much as it would have attacked an alien allograft.

We concluded from the cheek pouch experiments and the tail skin graft experiments that normal numbers of evenly distributed Langerhans cells were required to produce contact sensitivity to DNFB. Epidermis in which Langerhans cells were abnormal in 1 or both of these respects was incapable of supporting contact sensitization. However, epidermis containing an appropriate density of Langerhans cells could not, by itself, promote sensitization. The tissue also had to possess an intact lymphatic pathway for the regional dissemination of the immunogenic signal. When that pathway was blocked, as it appeared to be in the functionally intact hamster cheek pouch, induction of sensitivity failed to take place.

Alloantigenicity of Skin Grafts after Ultraviolet Light Irradiation

More than a decade ago, Steinmuller produced the first convincing evidence that nonparenchymal cells, i.e., cells of extracutaneous origin, contributed to the antigenicity of full-thickness skin allografts [12]. It has been assumed that these cells are derived from the peripheral blood, which delivers them to the skin. Therefore, the term "passenger cells" (or "passenger leukocytes") has been applied to them [13]. Their precise morphological identification has never been established. Perhaps more than 1 type of cell participates. It is not unreasonable to suggest that Langerhans cells, derived from mesenchyme, function in allografts as "passenger cells." We elected to examine this possibility by taking advantage of the fact that skin irradiated with UVL loses the vast majority of its ATPase-positive (Langerhans) cells [7] and fails to support the induction of contact hypersensitivity [6]. We hypothesized that UVL-treated skin might be relatively deficient not only in Langerhans cells but also in the unique cell surface (Ia) antigens they express.

In that regard, class I histocompatibility antigens (murine K/D) are expressed on virtually all cells of the body; class II antigens (murine Ia) are restricted in expression primarily to lymphoreticular cells [14,15]. In the epidermis, keratinocytes and Langerhans cells both express class I determinants, but only Langerhans cells express class II antigens [16,17]. We chose 6 inbred mouse strains (Table III) because they differed from each other only at the *K* region of H-2 (class I) or at the *I* region (class II). Skin graft donors received 4 daily exposures to UVL according to the schedule reported previously. After the terminal treatment, skin grafts were prepared from the irradiated areas and were placed on recipient mice. These grafts were monitored until rejection had taken place. Median survival times were calculated and compared with 1st-set controls whose grafts had received incandescent light treatment. Recipient

TABLE III. *H-2 genotypes of mouse strains employed*

Strain	H-2 regions			
	K	*I*	*S*	*D*
A.AL	k	k	k	d
A.TL	s	k	k	d
A.TH	s	s	s	d
B10.A	k	k/d	d	d
B10.AQR	q	k/d	d	d
B10.T(6R)	q	q	q	d

TABLE IV. *Effects of ultraviolet light irradiation on alloantigenicity of skin allografts*

Host	Donor	H-2 disparity	Graft survival[a]	
			1st set	2nd set
1. A.TL	A.AL-UVL[b]	*K*	12.5	8.3
2. A.TL	A.AL	*K*	11.5	9.0
3. A.TL	A.TH-UVL	*I*	11.6	8.5
4. A.TL	A.TH	*I*	12.4	8.5
5. B10.A	B10.AQR-UVL	*K*	11.4	8.8
6. B10.A	B10.AQR	*K*	11.5	8.1
7. B10.AQR	B10.T(6R)-UVL	*I*	12.5	10.5
8. B10.AQR	B10.T(6R)	*I*	11.0	10.0

[a] Median survival time in days.
[b] Abbreviation: UVL, ultraviolet light.

mice then received grafts of normal skin syngeneic with the donors of the UVL-treated skin. These 2nd-set grafts were also monitored until rejection and were compared with 2nd-set grafts on control animals. The results of these experiments are displayed on Table IV.

We did not expect class I antigens, expressed on keratinocytes, to be affected by UVL treatment, and they were not. The UVL-treated and normal grafts from A.AL and B10.AQR donors were rejected with equal vigor by A.TL and B10.A mice, respectively. If UVL treatment effectively removed Langerhans cells from the epidermis, UVL-treated grafts from A.TH and B10.T(6R) should have enjoyed prolonged survival on A.TL and B10.AQR recipient mice, respectively. They did not! Moreover, when these animals were rechallenged with normal A.TH and 6R skin grafts, respectively, they rejected the grafts in an accelerated manner, an indication that the 1st graft had immunized to class II (Ia) antigens of the donor.

It might be argued that the donor grafts in these experiments contained, among the passenger cell component, immunocompetent cells of the donor that were potentially able to react to host antigens and thus initiate within the graft bed itself a mild graft-versus-host reaction. To rule out this unlikely possibility, we carried out similar experiments with appropriate F_1 hybrid donors whose skin was irradiated with UVL. The results (not shown) were identical to those reported above. Even F_1 grafts treated with UVL sensitized their recipients to donor class II antigens.

There are 2 possible explanations for these results, either or both of which may be valid: (a) Langerhans cells were not the major contributors to the Class II alloantigenicity of the skin grafts and (b) UVL irradiation did not rid the epidermis of these cells (on the basis of grafting criteria).

Alloantigenicity of Cornea Grafts in Mice

Our efforts to deplete body wall epidermis *absolutely* of Langerhans cells were not successful. Therefore, we turned to grafts prepared from mouse cornea because of unequivocal evidence that this highly specialized epidermal tissue is devoid of Langerhans cells and other dendritic cells [4,18,19]. Corneas from donor mice were removed and their limbic attachments were carefully excised to ensure that tissue potentially laden with Langerhans cells was not included. Three such grafts, epidermal-side-up, were placed heterotopically on beds prepared on the thoracic walls of recipient mice. In preliminary experiments, syngeneic corneal grafts survived surprisingly well:

TABLE V. *First-set allografts: Cornea versus body wall skin*

Host	Donor	Antigen	Type	MST[a]
1. B10.AQR	B10.A	K^k	body wall	16.0
2. B10.AQR	B10.A	K^k	cornea	13.2
3. A.TH	A.TL	I^k	body wall	16.2
4. A.TH	A.TL	I^k	cornea	>45
5. A.TH	A.AL	K^kI^k	body wall	14.8

[a] Abbreviation: MST, median survival time in days.

TABLE VI. *Allograft immunity induced by cornea versus body wall skin*

Host	Sensitizing graft		2nd-set graft	
	Antigen	Type	Antigen	MST[a]
1. B10.AQR	K^k	body wall	K^k	9.0
2. B10.AQR	K^k	cornea	K^k	9.0
3. A.TH	I^k	body wall	K^kI^k	9.5
4. A.TH	I^k	cornea	K^kI^k	14.8
5. A.TH	K^kI^k	body wall	K^kI^k	9.0
6. A.TH		none	K^kI^k	15.0

[a] Abbreviation: MST, median survival time in days.

so long as a protective dressing of Vaseline-impregnated gauze was applied, they retained their transparency; when exposed to room air, the grafts became translucent but kept their dome-shaped configuration owing to the unique structure of the corneal stroma. Grafts such as these have been observed to remain in good condition for more than 60 days.

In experiments analogous to those just described for UVL-treated skin grafts, we tested whether corneal grafts between donor-recipient pairs disparate only for class II antigens would initiate specific allograft sensitization. The results of these experiments are displayed in Table V (1st set) and Table VI (2nd set). Corneal grafts expressed class I antigens quite well; they instigated their own specific rejection (line 2, Table V). By contrast, corneal grafts disparate from their recipients only for class II antigens were not rejected (line 4, Table V). When these recipients were rechallenged with body wall skin bearing class II antigens syngeneic with the corneal graft donors, the grafts were rejected in typical 1st-set fashion (line 4, Table VI), an indication that the original corneal graft had not immunized against these antigens. Importantly, corneal grafts disparate from their recipients for class I antigens not only were rejected, but also caused the development of 2nd-set reactivity when the animals were subsequently challenged with body wall skin of the same phenotype.

We concluded that the corneal grafts did not express Ia antigens in our *in vivo* functional assay. Moreover, since the corneal grafts were absolutely deficient in Langerhans cells but did have a resident population of other mesenchymal cells (including lymphocytes), we inferred that Langerhans cells were probably the major, if not the only, source of cells in the skin capable of expressing class II antigens in an immunogenetically important manner.

DISCUSSION

A vast body of evidence has accumulated over the past few years that Langerhans cells play a dominant role in the process by which antigens presented through cutaneous surfaces are perceived by the immunologic apparatus. Workers in several laboratories have contributed to this body of evidence. The watershed discovery was that Langerhans cells were not related to melanocytes [20], but represented a unique epidermal cell type, probably derived from mesenchyme [21]; then Langerhans cells began to become associated experimentally with contact hypersensitivity reactions [22,23]. The surface determinants and properties of these cells were found to closely resemble those of macrophages [16,24,25]; strong *in vitro* evidence implicated Langerhans cells in the important process of antigen presentation to immunocompetent lymphocytes [25]. In our own laboratory, we have studied Langerhans cells *in vivo* by using cutaneous surfaces containing aberrant densities and distributions of Langerhans cells and by manipulating Langerhans cells artificially with UVL and chemical irritants. We have been rewarded with an illuminating series of findings. First, cutaneous sites with unique immunologic properties have fewer Langerhans cells than normal skin sites, and the distribution of cells is aberrant. Second, Langerhans cells and their influence can be effectively depleted from skin by exposure to UVL. Third, anatomically intact skin deficient in Langerhans cells does not promote the induction of contact hypersensitivity. Fourth, heterotopic grafts of skin deficient in Langerhans cells do not permit contact sensitization, an indication that the failure is due to an inherent property of that skin. Fifth, epidermis absolutely devoid of Langerhans cells cannot express, in an immunogenically active form, class II antigens of the major histocompatibility complex, even though other lymphoreticular cells may be present in the graft. Sixth, and most importantly, cutaneous surfaces depleted of Langerhans cells promote the development of specific immunologic tolerance to antigens painted thereon.

We have not yet fulfilled Robert Koch's famous postulates as they pertain to Langerhans cell function, that is, we have not reconstituted a putative Langerhans cell-dependent function by the addition of exogenous Langerhans cells. The closest we have come has been in the corneal allograft experiments, in which we have become absolutely sure that the grafts being used are devoid of Langerhans cells. Until a successful method for preparing cell suspensions selectively purified for Langerhans cells has been devised, the amassed evidence must remain circumstantial.

We are also cautioned by the observations made with cheek-pouch-grafted hamsters. In our enthusiasm to assign a pivotal role to Langerhans cells in the induction of immunity to chemical contactants, we transiently overlooked the much older observation of Barker and Billingham that cheek pouches lacked a lymphatic drainage pathway [8]. The results of our experiments remind us that fundamentally we know nothing about the transduction of "antigen" into an immunogenic signal. Do Langerhans cells, once derivatized with hapten, drop down through the dermal-epidermal junction and travel through the afferent lymphatics to present the antigen at the draining node? Or is the signal somehow imparted to recirculating T lymphocytes passing through the epidermis, and must these cells then traffic back to the draining node? Could it be that the draining lymphatic is not important because of the strategic location of the regional lymph node? Perhaps immunologic recognition by T lymphocytes occurs within the epidermis itself, upon a substrate prepared by antigen-derivatized Langerhans cells, and the activated T cells must gain access to the systemic circulation through the lymphatic route.

With this last possibility, our attention returns to the hypothesis that an integrated system of skin-associated lymphoid tissue (SALT) exists [13]. The results of our experiments could be interpreted to mean that in the constellation of tissues and cells that constitute SALT, Langerhans cells are the anatomical outposts within the integument, and that recirculating lymphocytes become specialized by developing "affinities" for Langerhans cells, thus accounting for their apparent attraction to skin. As sentinels within the epidermis, Langerhans cells provide a continuous network of dendritic processes for the efficient capture and processing of cutaneously presented antigen. Effective presentation of antigens to wandering skin-specific lymphocytes becomes their major physiological role. Where the Langerhans cell network is attenuated or absent, e.g., the cornea, one might expect the cutaneous surface to be especially vulnerable immunologically and to permit antigens presented at that site to be perceived as tolerogens rather than as immunogens. We can only speculate upon the possibility that the recurrent, devastating keratitis that follows corneal infection with herpes sim-

plex virus might be an example of this phenomenon.

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REFERENCES

1. Bergstresser PR, Toews GB, Streilein JW: Natural and perturbed distributions of Langerhans cells: Grafting and dinitrochlorobenzene sensitization. J Invest Dermatol 75(1): this issue
2. Toews GB, Bergstresser PR, Streilein JW: Langerhans cells: Sentinels of skin associated lymphoid tissues. J Invest Dermatol 75(1): this issue
3. Billingham RE, Silvers WK: Free skin grafting in mammals, Transplantation of Tissues and Cells. Edited by RE Billingham, WK Silvers. Philadelphia, Wistar Institute Press, 1961, pp 1–26
4. Streilein JW, Toews GB, Bergstresser PR: Corneal allografts fail to express Ia antigens. Nature 282:326–327, 1979.
5. Litchfield JT Jr, Wilcoxon F: Nomographic method for calculation of median survival times. J Pharmacol Exp Ther 96:399–408, 1949
6. Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact sensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
7. Bergstresser PR, Fletcher C, Streilein JW: Surface densities of Langerhans cells in relation to rodent epidermal sites with special immunologic properties. J Invest Dermatol 74:77–80, 1980
8. Barker C, Billingham RE: Immunologically privileged sites. Adv Immunol 25:1–54, 1977
9. Streilein JW: Contact hypersensitivity, humoral immunity and specific unresponsiveness can be induced in Syrian hamsters with simple haptens. J Immunol, 124:577–585, 1980
10. Chase MW, Battisto JR: Immunological unresponsiveness to allergenic chemicals, Mechanisms of Hypersensitivity (Henry Ford Hospital International Symposium). Edited by JH Shaffer, GA LoGrippo, MW Chase. Boston, Little Brown and Company, 1959, pp 507–517
11. Chase MW: Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med 61:257–259, 1946
12. Steinmuller D: Immunization with skin isografts taken from tolerant mice. Science 158:127–129, 1967
13. Streilein JW: Lymphocyte traffic, T-cell malignancies and the skin. J Invest Dermatol 71:167–171, 1978
14. Klein J: Biology of the Mouse Histocompatibility-2 Complex. New York, Springer-Verlag, 1975
15. Shreffler DC, David CS: The H-2 major histocompatibility complex and the I immune response region: Genetic variation, function and organization. Adv Immunol 20:125–195, 1975
16. Rowden G, Phillips TM, Delovitch TL: Expression of Ia antigens by murine keratinizing epithelial Langerhans cells. Immunogenetics 7:465–478, 1978
17. Tamaki K, Stingl G, Gullino M, Sachs DH, Katz SI: Ia antigens in mouse skin are predominantly expressed on Langerhans cells. J Immunol 123:784–787, 1979
18. Redsllob E: Etude sur le pigment de l'epithelium conjunctival et corneon. Ann Oculist (Paris) 159:523–537, 1922
19. Pei YF, Rhodin JAG: Electron microscopic study of the development of the mouse corneal epithelium. Invest Ophthalmol 10: 811–825, 1971
20. Breathnach AS, Silvers WK, Smith J, Heyner S: Langerhans cells in mouse skin experimentally deprived of its neural crest component. J Invest Dermatol 50:147–160, 1968
21. Billingham RE, Silvers WK: Some unsolved problems in the biology of skin, Biology of the Skin and Hair Growth. Edited by AG Lyne, BF Short. New York, American-Elsevier, 1965, pp 1–24
22. Silberberg I: Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. Acta Derm Venereol (Stockh) 53:1–12, 1973
23. Shelley WB, Juhlin L: Langerhans cells form a reticuloepithelial trap for external contact antigens. Nature 261:46–47, 1976
24. Stingl G, Wolff-Schreiner EC, Pichler WJ, Schnait F, Knapp W, Wolff K: Epidermal Langerhans cells bear Fc and C3 receptors. Nature 268:245–246, 1977
25. Stingl G, Katz SI, Clement I, Green I, Shevach EM: Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 121:2005–2013, 1978